

Figure #1

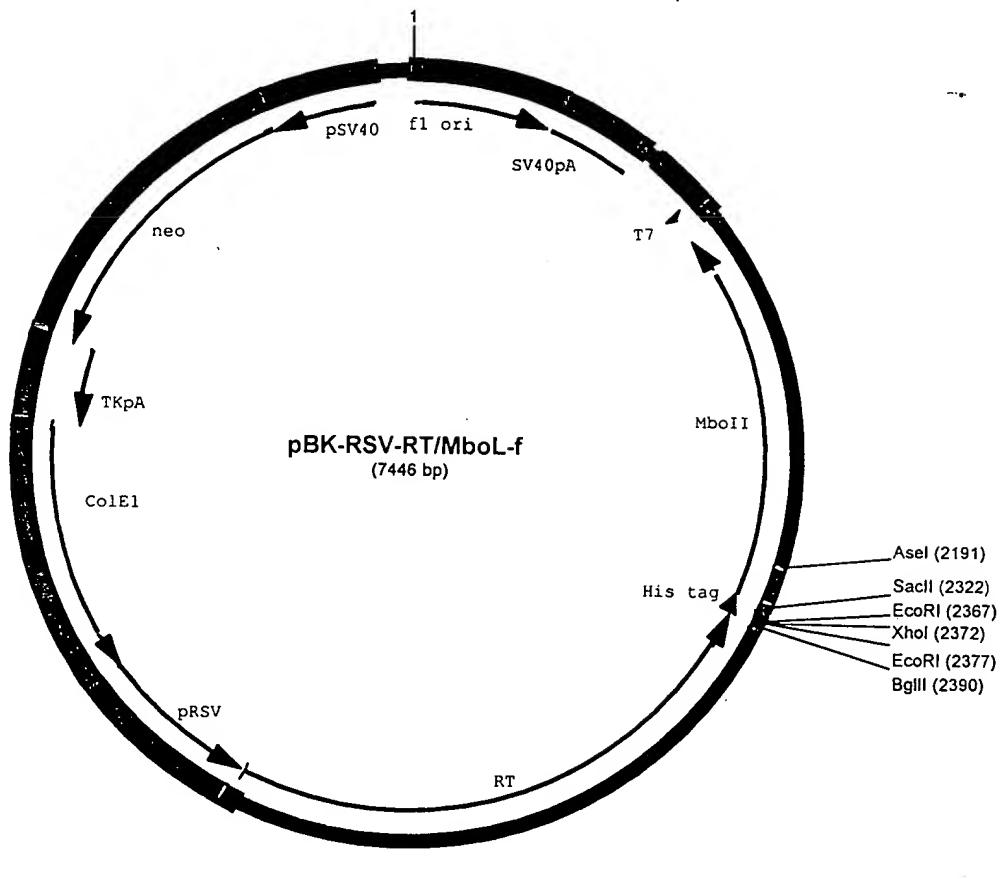


Figure 1: Structure of the pssDNA-Express A plasmid. The reverse transcriptase and *Mbo* II genes are expressed in a single polypeptide for which the message is transcribed from the RSV promoter. The reverse transcriptase and *Mbo* II domains are separated by a histidine- and proline-rich linker.

Figure #2

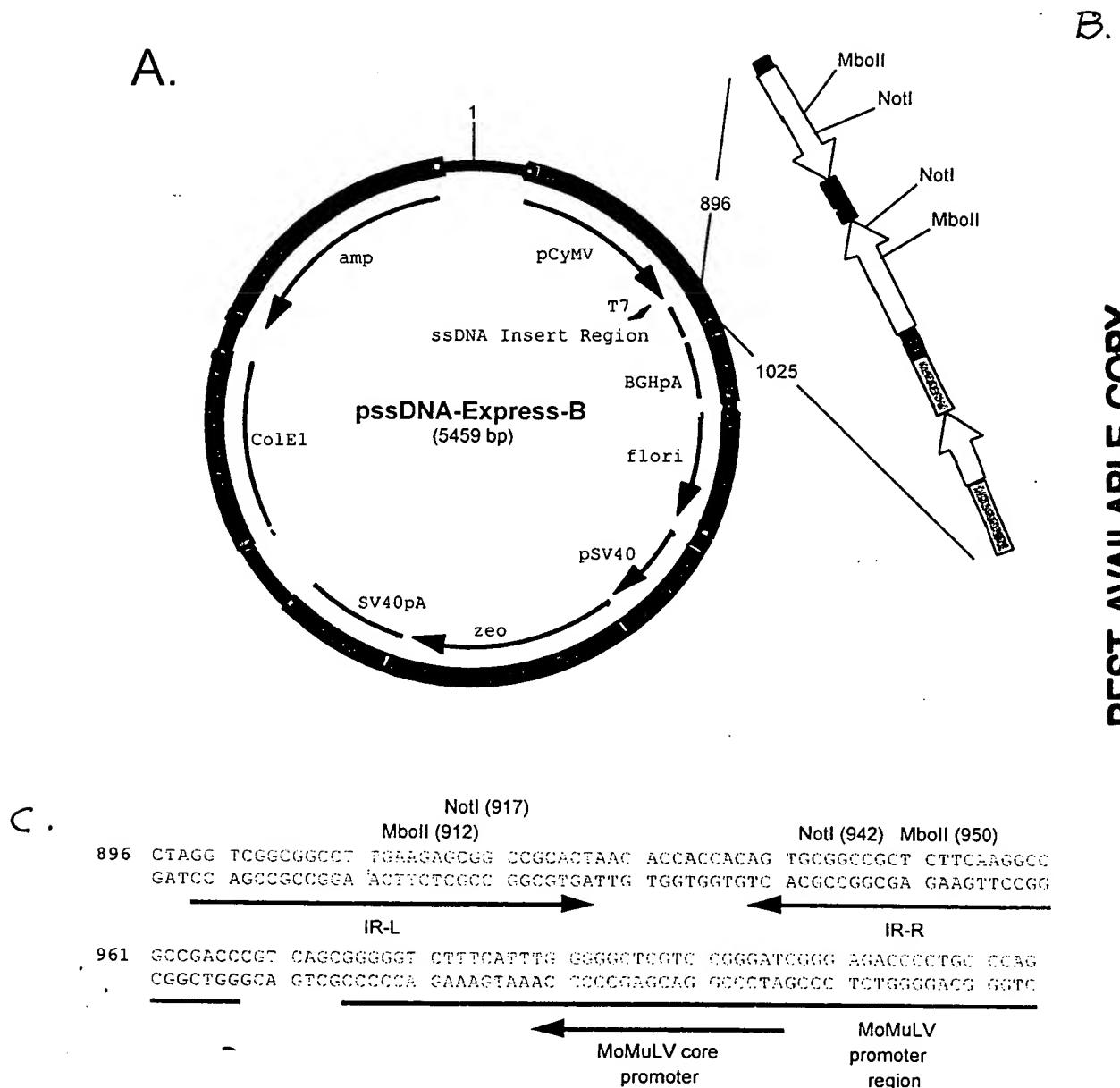


Figure 2: Structure of the pssDNA-Express-B plasmid. A. Plasmid map with enlargement showing arrangement of insert features: inverted complementary repeats (open arrows); MoMuLV promoter region (hatched) and MoMuLV core promoter (stippled arrow). B. Sequence of the insert region.

Figure #3

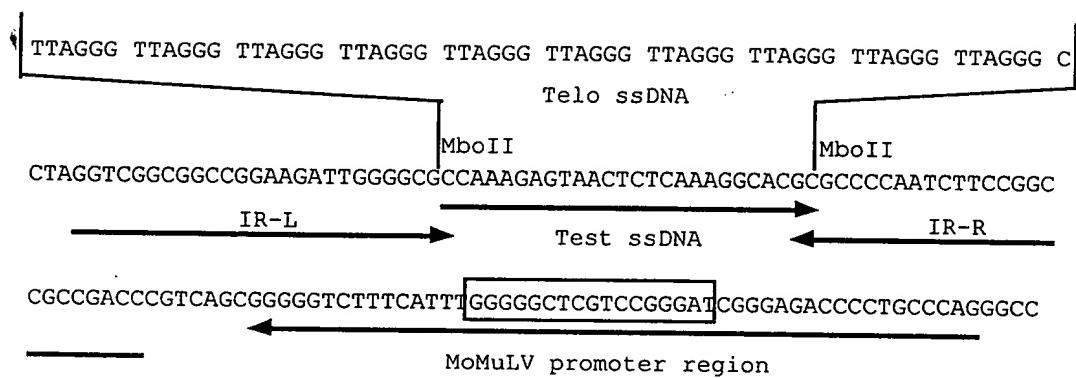


Figure #4

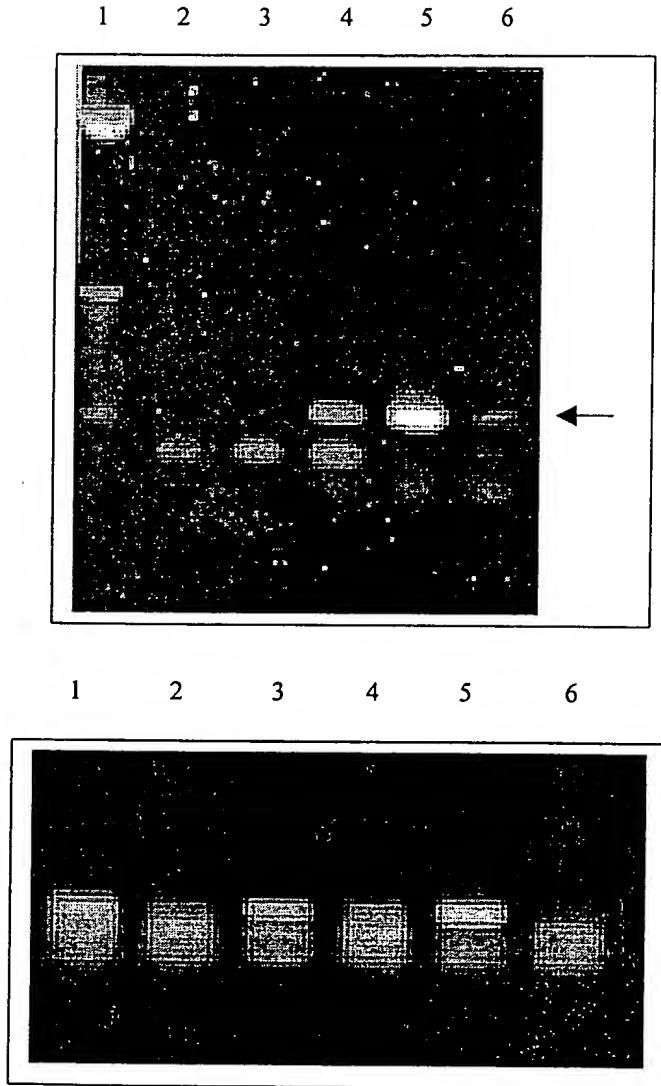


Figure 4: Reverse transcriptase assays on RNA/ssDNA extracts from transformed HeLa cell lines. A. Transient transformations. Lanes: (1) size markers; (2) untransformed HeLa cells; (3) HeLa cells transformed with the vector pcDNA3.1Zeo⁺; (4) HeLa cells transformed with pssXA; (5) positive control containing 2 million units of MoMuLV reverse transcriptase; (6) negative control containing no protein extract. The arrow indicates the 150 bp amplification product. (Bk2p106)

B. Reverse transcriptase assays on stably transformed cell lines. Lanes: (1) clone A12, (2) B8, (3) B12, (4) D7, (5) positive control containing 2 mU of MoMuLV reverse transcriptase, (6) Control containing no extract. (Bk2p152)

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Figure #5

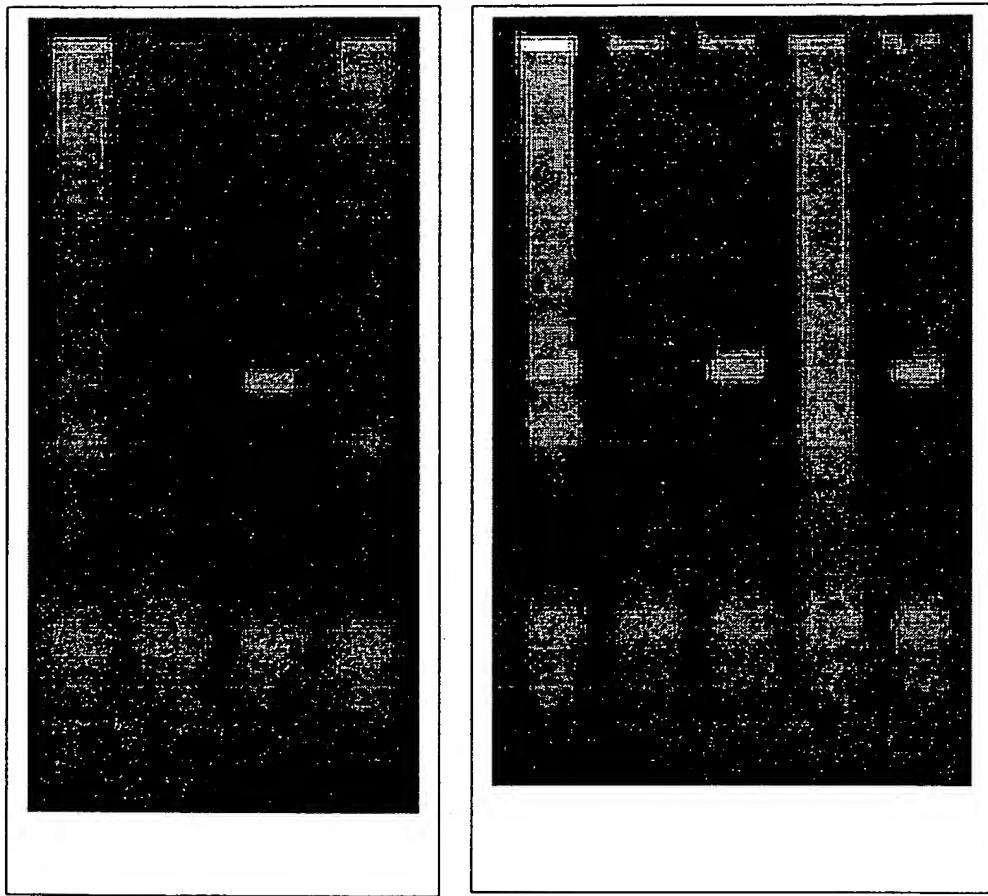


Figure 5: Amplification of single-stranded DNA from extracts of transformed cells.
RNA/ssDNA preparations were used as templates in PCR reactions using primers specific to the expected ssDNA product. Left panel, lanes 1-3: total RNA/ssDNA PCR template isolated from stably transformed colony A12 (1) used with no prior treatment (2) treated with S1 nuclease, (3) treated with RNase A. Lane (4) total RNA/ssDNA from a colony stably transformed with the pcDNA3.1Zeo+ vector alone. (Bk3p12a lanes 5-8) Right panel, lanes 1-3: total RNA/ssDNA PCR template isolated from stably transformed colony B12 (1) used with no prior treatment (2) treated with S1 nuclease, (3) treated with RNase A. Lane (4), total RNA/ssDNA from a colony stably transformed with the pcDNA3.1Zeo+ vector alone. Lane (5), positive control template, plasmid pTest.

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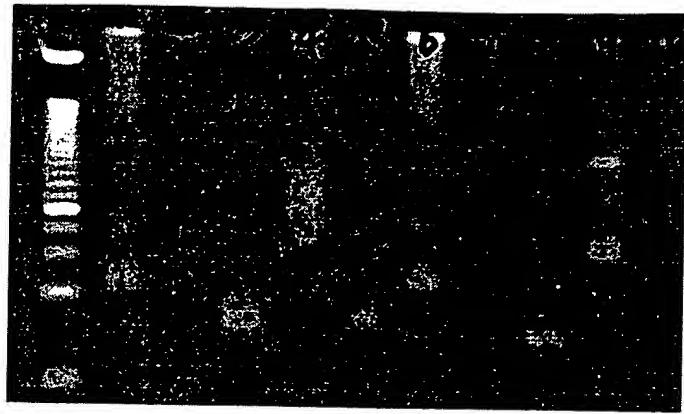
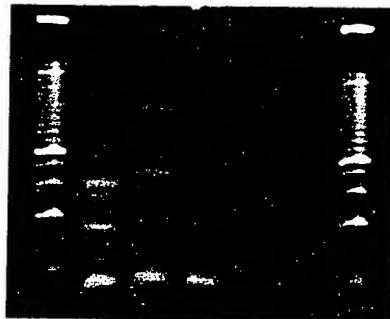


FIG. 6

1. 2. 3. 4. 5. 6. 7.

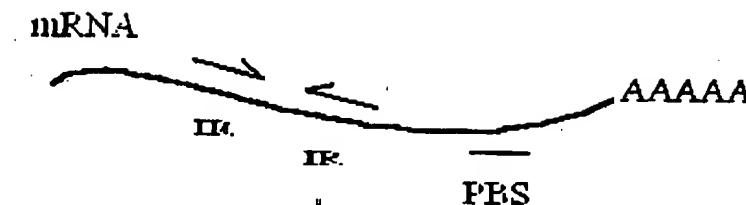
Extension loop-
Stem juncture-



25 b.p.
ladder

FIG. 7

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mRNA folds to stem-loop Intermediate

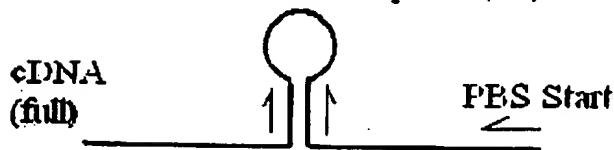
cDNA start

AAAA

tRNA binds to PBS and primes cDNA

cDNA Conversion

Stem-loop Structure
from Inverted Repeats (IR)



Or

cDNA Pre-mature
Termination from Stem-loop

PBS Start

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